

Review

Pros and Cons of Human Brain Organoids to Study Alzheimer's Disease

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ABSTRACT: There is increasing pressure for researchers to reduce their reliance on animals, particularly in early-stage research. The main reason for that change arises from the different biological behavior of humans that leads to frequent failure of translating data from bench to bed. The advent of organoid technology ten years ago, along with the feasibility of obtaining brain organoids in most laboratories, has created considerable expectations not exempting frustration. In this review, we make a critical appraisal of the advantages and limitations of studying Alzheimer's disease in brain cortical organoids derived from inducible pluripotent stem cells (iPSCs). While dealing with human neurons and glia in 3D poses a tremendous advantage versus murine brain cells, organoids typically lack microglia, blood vessels, immune interactions as well as proper CNS neuropil. In turn, they have relatively few oligodendrocytes and low myelination. In addition, lengthy procedures to get proper mature organoids constitute an additional limitation that may also affect the native biological properties of neurons and glia. We conclude that human brain organoids, while popular and useful, remain a model that needs further refinement before bringing substantial value to study Alzheimer's disease.

Key words: Alzheimer's disease, neurodegeneration, cerebral organoids, therapy

SEARCH AND SELECTION STRATEGY

The bibliographic search strategy was carried out in August 2024, using the following bioethical and biomedical databases: PubMed, Medline, Web of Science Core Collection, and Cochrane. To guide the search, we establish a series of tools or search terms in accordance with the agreed theme. Through an indexing process, the free terms were replaced by standardized and unambiguous terms (MeSH terms) targeting 'Organoids' and 'Alzheimer's disease'. The logical relationship between terms was achieved using Boolean operators and truncations to retrieve those articles that had the same grammatical root in the databases that made the option possible. Articles in English were considered for full text analysis. We excluded abstracts for conferences and

societies. All articles that meet our criteria are listed in the References section.

1. Brain organoids recreate the complex cellular diversity present in the human brain

Since Alzheimer's disease (AD) is the most prevalent neurodegenerative disease of the Central Nervous System (CNS), there has been considerable effort in using neural organoid models to study this disorder. Organoids, in general, are the result of an innate ability of specific cell types to self-organize and emulate the fundamental functional properties and complex structure of various organs or tissues [1–5]. The result is a miniature in vitro version of a human organ, characterized by many similarities both in its architecture and in physiology,

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albeit with significant differences too, with respect to the original organ. Despite the valuable opportunities that animal models have provided for translational discoveries, it is important to recognize the intrinsic limitations stemming from differences in physiology compared to humans, which human organoids attempt to transcend. Earlier work using two-dimensional (2D) tissue or human and animal cell culture models have also provided novel insights into aspects of disease biology. However, these models tend to be static and simplified and have lack the ability to replicate the dynamics and complexity of biological processes *in vivo*. These restrictions have encouraged the urgent need to develop *in vitro* platforms that are more dynamic and deeply complex.

In this review, we will discuss the complex cellular interactions involved in AD as well as the main advantages and disadvantages in the use of three-dimensional (3D) human cell culture models taking into account the most recent advances in the creation and development of various neural organoid models.

2. The biology of neurons and glial cells and their complex roles in AD

AD is the most prevalent neurodegenerative disorder globally, with aging being the primary risk factor. It is characterized by a gradual loss of mnemonic and cognitive function because of the abnormal agglomeration of amyloid beta (A β) plaques and tau hyperphosphorylation-derived neurofibrillary tangles (NFTs), leading to neuronal demise and brain atrophy [6–9].

The finding of amyloid plaques isolated from individuals with AD and Down syndrome (DS) in the mid-1980s placed A β protein as a key event in disease pathophysiology [10–12]. This connection fueled the conjecture that the gene that encodes the A β protein might reside on chromosome 21, given that full trisomy of chromosome 21 had been found to play a role in the genesis of DS. Likewise, DS brains display an early pathology of amyloid plaques, culminating in dementia in most cases around 50 years of age [13,14]. This hypothesis gained ground when it was revealed that the amyloid precursor protein (APP) gene, which encodes a larger type 1 transmembrane protein, from which the A β peptide is cleaved, was located on chromosome 21 [15,16]. These early milestones in AD research laid the foundation for the amyloid cascade hypothesis [17]. This hypothesis postulates that A β accumulation or deposition is the initial event in AD pathogenesis, triggering subsequent correlated pathophysiological events. These events include NFTs, synaptic dysfunction, inflammation, neurodegeneration, vascular aberrations, and finally

dementia [17,18]. This predominant framework has driven research toward therapeutic strategies aimed at reducing A β production and aggregation. It has also inspired efforts to enhance A β clearance and ultimately decrease amyloid plaques. However, while the amyloid cascade hypothesis for AD has been pivotal in advancing research on the biological and pathological roles of A β , it has faced increasing criticism within the scientific field over the last decades [19]. On the other hand, the lack of success in clinical trials focusing on A β -targeted drugs has prompted the scientific community to explore alternative disease pathways and therapeutic approaches related to tau. Furthermore, the presence of NFTs is more closely related to clinical symptoms and neuronal loss [20,21].

In addition to A β and tau components, emerging evidence indicates that other neuronal stressors, such as neuroinflammation, play a role in the development of AD. The presence of reactive astrocytes and elevated levels of proinflammatory molecules occur throughout the progression of the disease. Astrocytes play a fundamental role in regulating neuronal connections by facilitating communication between pre and postsynaptic neurons [22]. They are considered essential components for promoting long-term potentiation (LTP), managing neural energy, and releasing signaling substances called gliotransmitters [23]. These cells also undergo changes in both their structure and function in response to diseases and abnormal conditions in the brain [24–26]. These changes trigger a response in astrocytes, the main purpose of which is to protect the brain from damage during the early stages of the disease. However, this response can have detrimental effects in more advanced stages of the disease [27,28]. In this regard, the beneficial properties of astrocytes are compromised as the gliosis reaction continues to evolve and progress. A prolonged state of astrocytic activation leads to an increase in the release of inflammatory cytokines and the promotion of an environment conducive to neuronal damage, while they lose their neuronal support and nutrition functions [29]. Notably, astrocytes derived from iPSCs of familial (FAD) and sporadic (SAD) AD patients display an autonomous pathological phenotype that includes atrophy suggesting a relevant role of astrogliosis in the development of AD [30].

Oligodendrocytes (OLs) also hold a genuine interest in the pathological context of AD, considering their role in the generation of myelin and support for axons [31]. Alterations in white matter (for example, lesions, volumetric decrease, microstructural detriment) and the demyelinating process have been widely reported in the context of the aforementioned pathological entity [32].

The research has also revealed significant data from whole exome sequencing studies, which have implicated the innate immune system as a critical element in the

pathogenesis of AD. Recent discoveries have identified rare variants in the TREM2 gene (Triggering Receptor Expressed on Myeloid Cells 2), which is expressed in microglia. These variants increase the risk of developing AD by 2- to 4-fold, a risk level comparable to that conferred by the apolipoprotein E4 allele [33–35]. Thus, the expression of TREM2 in microglia appears to regulate the ability of these cells to engulf and eliminate cell debris, as well as their response to brain insults [36,37]. This highlights the role of TREM2 in modulating the neuroinflammatory processes that drive AD pathogenesis. Furthermore, ongoing accumulating evidence suggests that TREM2 activity is closely linked to lipid metabolism in microglia [38]. A recent analysis found that Trem2^{-/-} microglia in transgenic mice exhibit an inability to amplify transcripts associated with activation, lipid catabolism, and phagocytosis in response to myelin injury. This results in inefficient elimination of myelin residues, axonal dystrophy and long-lasting demyelination [39]. A number of AD susceptibility genes, apart from TREM2, also display preeminent or selective expression in microglia, e.g., CD33, INPP5D, MS4A6A and PLCG2. Therefore, it is plausible that they may influence the same activities and pathways regulated by TREM2 [40,41].

Finally, brain health depends on the vasculature of the brain, another fundamental element of great importance in this neurodegenerative disease [42]. The specialized features of blood vessels in the brain originate from a complex cellular community that interacts in a coordinated manner creating the blood-brain barrier (BBB). Among these cells are endothelial cells (ECs), which line the interior of the vessels; smooth muscle cells, surrounding the vessel; the pericytes, in close relationship with the basal membrane; immune cells near the vessels; and astrocytes surrounding the structure [43,44]. This cellular heterogeneity facilitates selective and hemodynamically sensitive transport of molecules between the bloodstream and the brain [45,46]. Consequently, BBB dysfunction could allow access of toxic molecules and cells from the blood, triggering an immune response related to neuroinflammation. This chain of events leads to degeneration of the neurovascular unit [47]. Likewise, the deterioration of the blood-cerebrospinal fluid (CSF) barrier, located in the choroid plexus (ChP) of the cerebral ventricles, due to the presence of A β , causes capillary damage and interstitial fibrosis, contributing to cerebral neurotoxicity [48]. In addition, alterations in the composition and production of CSF have been described. This is due to a reduction in the capacity of the ECs of the ChP to synthesize essential proteins required for CSF production. A decrease in the expression of ion transporters involved in its secretion has also been reported [49–52]. All of these dysregulations

converge to accelerate neural network degeneration, nerve cell loss, and ultimately cognitive decline.

These intricate and dynamic interactions between cell varieties are factors that contribute to the progression of the disease. This underscores the need to explore a diversity of approaches that lead to a range of effective therapies for this neurodegenerative condition.

3. Neural divergences across species: A comparative perspective

Transcriptomic and epigenomic analysis methods at the single-cell level have proven effective in unraveling the cellular composition of complex brain tissues by examining gene expression patterns and underlying regulatory mechanisms.

Transcriptomic analysis of various regions particularly relevant for exploring cellular evolution in rodents and primates, has revealed a remarkably conserved cellular composition across the brain. However, despite this general conservation, species-specific adaptations are also evident, including differences in cell type proportions, gene expression, DNA methylation, and chromatin state [53]. Thus, the temporal cerebral cortex of individuals with AD shows a significant alteration in the expression of 100 genes in comparison to unaffected individuals. These genes have previously been identified as risk factors for AD through genome-wide association studies (GWAS). However, mice carrying a specific mutation in the APP gene showed 27 genes whose expression was significantly altered in the temporal cortex [54]. This divergence is especially notable in non-neuronal cells, given that human microglia express the vast majority of genes related to susceptibility to AD, while rodent microglia only express a fraction of these genes [55]. Along with microglia, brain ECs and pericytes exhibit the greatest transcriptional divergence [56,57]. Disparity in gene expression profiles is a result of evolution and may contribute to phenotypic differences between species. Indeed, human astrocytes exhibit larger size and more complex morphology compared to their mouse counterparts [58,59]. Likewise, differences have been discerned in astrocytic metabolism, susceptibility to oxidative stress, attributable to variations in mitochondrial physiology and detoxification pathways, and in molecular responses under inflammatory conditions between both species [60]. Molecular differences between OLs of different species have also been the subject of research. Proteomic analyzes of purified myelin reveal that there is a certain degree of divergence in the protein composition of human and mouse CNS myelin [61,62].

Moreover, some primate species can spontaneously develop A β plaques and NFTs as they age [63–66].

Remarkably, the initiation and propagation of tau pathology in these primates closely mirror the patterns observed in AD patients. This finding strongly indicates that monkeys provide an unparalleled physiological model for investigating late-onset SAD. Environmental toxicants might contribute to AD pathogenesis, exemplified by the study conducted by Yang et al. [57], which utilized methanol administration to induce an AD model in monkeys. Furthermore, various research efforts have endeavored to create AD models through

intracerebral or lateral ventricular injections of synthetic or patient-derived Aβ oligomers [67–70]. These approaches have led to early pathological manifestations, such as dendritic spine reduction, heightened inflammation, and synaptic dysfunction, as well as widespread Aβ accumulation and hyperphosphorylation of tau, the microtubule-associated protein (P-tau) [67–69]. However, despite these findings, their use involves significant ethical concerns (Fig. 1) [58,71–77].

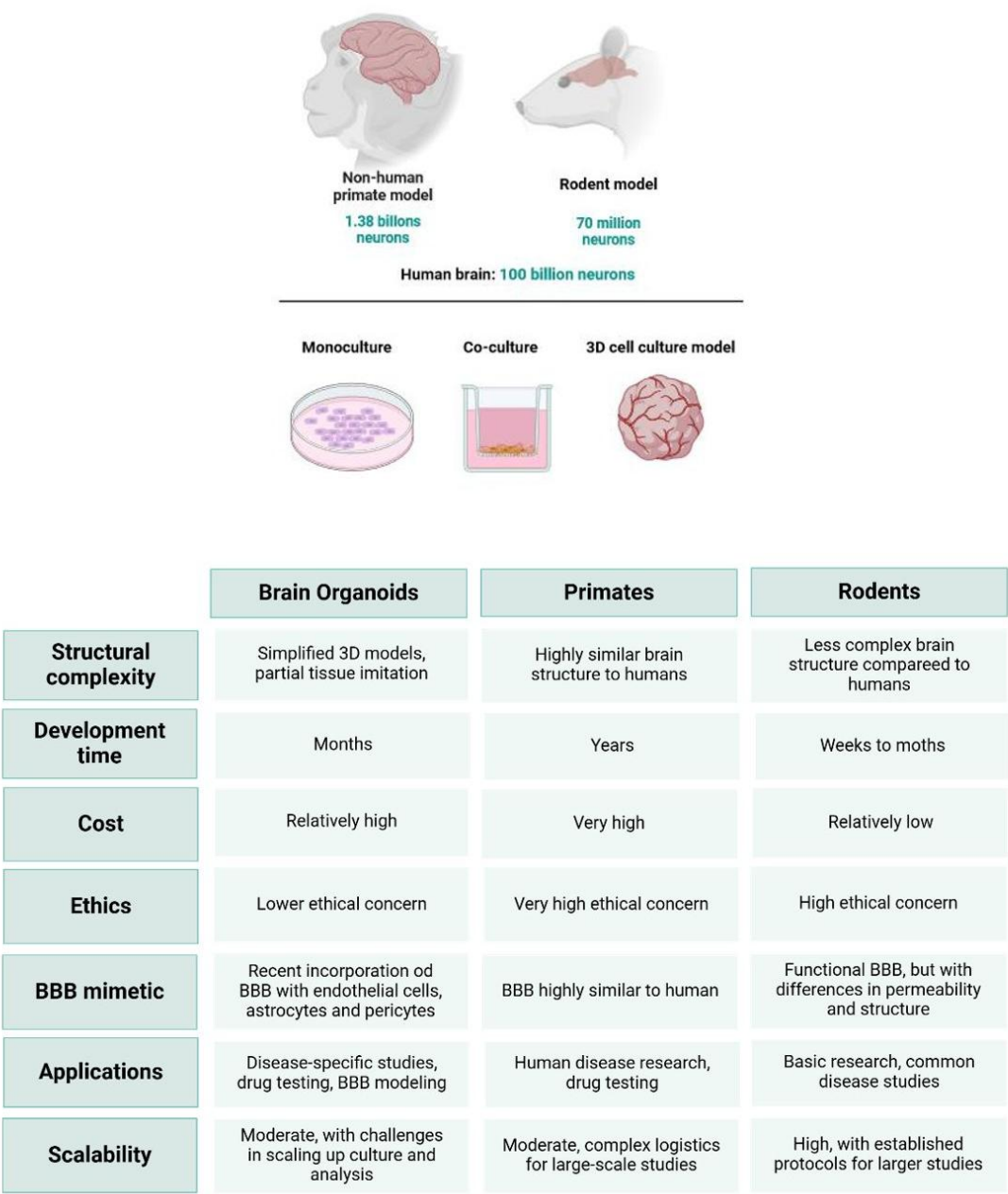


Figure 1. Comparison of brain organoids and animal models: Overview of structural complexity, blood-brain barrier (BBB) mimicry, cost, ethics, applications and scalability [71–77]. Created with BioRender.com.

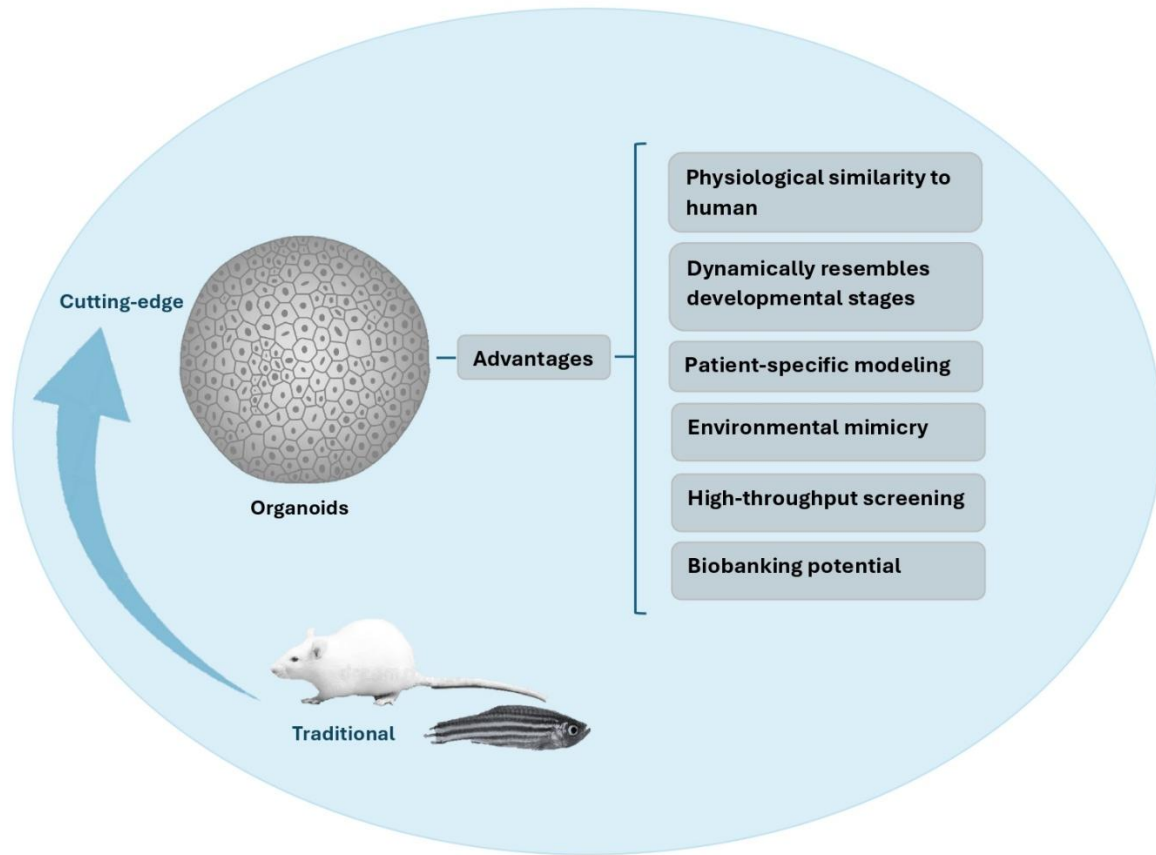


Figure 2. Leveraging brain organoids: Pivotal advantages in modeling neurodegenerative diseases. Brain organoids currently stand as the most sophisticated in vitro model for studying human brain development and disease, bridging the gap between basic science and clinical applications. Their ability to mimic the intricate cellular composition and physiological processes of the human brain allows for a more accurate representation of disease mechanisms, particularly for neurodegenerative conditions like AD and Parkinson disease (PD). Importantly, brain organoids recapitulate the complex process of human cortical development, including the characteristic organization of the progenitor zone with abundant external radial glial stem cells and the epigenomic signatures of the human fetal brain [78, 79]. Single-cell RNA sequencing analysis comparing brain organoids to the fetal neocortex has revealed that organoids in vitro closely mimic human neocortex development [80]. These findings strongly support the utility of human brain organoids in studies of human development. In comparison to traditional 2D cultures and animal models, organoids offer a more relevant and scalable platform for high-throughput drug screening, enabling the identification of potential therapeutic candidates in a human-like context [81]. The development of patient-specific organoids derived from iPSCs has further advanced the field, allowing researchers to explore disease variability at an individual level and opening the door to personalized medicine. Moreover, organoids are becoming integral components of biobanks, providing valuable resources for large-scale studies on disease progression, treatment efficacy, and cellular behavior [82]. Despite certain limitations, organoids remain a promising and versatile tool that overcomes many of the constraints posed by alternative models, significantly enhancing our ability to study human-specific neurobiology. Created using Microsoft.

These species-dependent divergences represent a barrier in translational research, as more than 90% of neurological drug candidates that show promising results in animal studies fail in human clinical trials. Organoids from patients with diseases reproduce the cellular phenotypes related to the pathology under study, providing the opportunity to elucidate the outcome of the disease in different tissues. In addition, they allow susceptibility testing to specific drugs and the creation of personalized therapeutic strategies (Fig. 2).

4. Organoid derivation methods and their limitations.

Pluripotent stem cells, namely, embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), have recently been explored for clinical use due to their remarkable ability to differentiate into cells of all three embryonic lineages [83]. The main concerns about ESCs involve ethical issues linked to the use of human embryos and the possibility of post-transplant immunological rejection. These conflicts have driven progress toward

cellular reprogramming. In 2006, a team of scientists led by Shinya Yamanaka achieved a milestone by obtaining pluripotent stem cells from mouse fibroblasts [84]. Shortly after, in 2008, Yamanaka's team successfully replicated the same process using human cells [85]. The initial protocol used to obtain the first iPSCs from fibroblasts consisted of the combination of 4 transcription factors known as the “Yamanaka cocktail”, KLF4, SOX2, OCT4 and cMYC, encoded in a retrovirus. Over-expression of these factors had the ability to reverse the differentiation of somatic cells and reprogram them back into pluripotent stem cells [86]. However, the risk of tumorigenesis implied by the introduction of the c-Myc oncogene and its permanent integration was an obstacle to its clinical application [87]. To date, various groups have emphasized the potential of this strategy by optimizing the process to increase its efficiency by excluding this transcription factor [88,89]. The establishment of other messenger RNA-based, episomal vectors, transient viral infections or protein reprogramming methods has also been considered for the generation of human iPSCs without permanent genetic alterations [90]. Differentiated iPSCs routinely achieve gene expression profiles that directly match various brain cell types present in the appropriate *in vivo* environment. Of particular significance, 2D cultures derived from iPSCs have emerged as indispensable tools for elucidating intricate cellular behaviors and pathophysiological mechanisms within a controlled experimental framework. These 2D systems allow for precise manipulation of the extracellular environment, facilitating the study of neuronal differentiation, synaptic formation, and the progression of neurodegenerative diseases [91]. iPSC-derived neurons cultured in 2D have been successfully employed to model AD, revealing key insights into A β plaque formation and tau protein aggregation [92–99]. Nevertheless, 2D monolayer platforms are inherently limited in their capacity to replicate the complex three-dimensional architecture of the brain. They lack the tissue-specific spatial and morphological cellular organization, essential cell-cell and cell-extracellular matrix (ECM) interactions, as well as the inclusion of immune system cells and the dynamics of waste accumulation and clearance, which are crucial for accurately modeling physiological and pathological processes [100,101].

In recent years, various attempts have been made to model specific brain structures more faithfully by employing organoids, which represent a significant advancement over traditional 2D cultures. A crucial question in optimizing organoid creation methods is whether to deliberately promote cell differentiation toward specific fates. This can be addressed by the presence or absence of exogenous morphogens and

signaling molecules in the stem cell medium. Protocols that promote spontaneous differentiation by avoiding the addition of external factors to the environment lead to the generation of heterogeneous cell populations. These populations correspond to various areas of the brain, spanning from the retina to the hindbrain [102].

The research group led by Sasai pioneered the generation of brain organoids, using methods that guided differentiation towards the forebrain and protocols to obtain optic cups [103]. They were based on the cultivation of stem cell aggregates under serum-free conditions. This 3D aggregation system, which they named SFEBq (serum-free floating culture of embryoid body-like aggregates with rapid reaggregation), enabled the self-organized formation of mouse and human embryonic stem cell aggregates [104]. This was achieved by the addition of a molecule that generated gradients of intra-aggregate morphogens, such as the treatment aimed at promoting the rostral pallial region through FGF8 [103,104]. Thus, guided differentiation protocols encompass an initial phase of stem cell aggregation, a general neural induction stage, and, in numerous cases, short periods of exposure to specific morphogens to direct the formation of particular tissue patterns followed by a prolonged period of stimulation with growth factors.

Lancaster et al. made significant strides in the field with their groundbreaking work on cerebral organoids [79]. These early efforts led to the development of organoids exhibiting various regional identities, organized as distinct domains capable of influencing one another (Fig. 3). In their seminal work, Lancaster et al. pioneered the development of brain organoids with a forebrain-like architecture by employing poly(lactide-co-glycolide) (PLGA) copolymer fiber microfilaments as a floating scaffold for the generation of elongated embryoid bodies (EBs). This is because most brain organoid culture procedures start from spherical structures or EBs, where the developing nervous tissue grows on the periphery of the EB. Lancaster and colleagues hypothesized that the variability in neural ectoderm formation could arise due to the unfavorable relationship between surface area and volume. Analysis using EB staining for specific markers of the germ layers, in early stages, revealed the generation of a more uniform and reproducible polarized neuroepithelium with reduced amounts of endodermal and mesodermal tissue. This advancement demonstrated a consistent ability for developing nervous tissue without compromising the organoid's self-organization, leading to improved neuronal induction of the forebrain [105]. Despite these advances, undirected differentiation remains a challenge, with some cells adopting non-ectodermal fates. Consequently, ongoing research focuses on refining protocols to optimize the application of external signals to direct differentiation towards neurons

[78,106]. The establishment of the foundations of the field paved the way for other scientific teams to develop organoids with specific regional destinations (spinal cord, basal ganglia, hippocampus, cerebellum, etc.) [107–110]. One of the challenges in the field of organoid research lies in the use of ECMs derived from animals, such as Matrigel used in the initial protocol. Matrigel is derived from the ECM secreted by Engelbreth-Holm-Swarm mouse sarcoma cells and is enriched in ECM proteins [111]. Despite its versatility and affordable cost, Matrigel is extremely complex. The undefined nature of this matrix can complicate the identification of signals essential for organoid structure and function, compounded by batch-to-batch variations of this material [112]. Aspects such as the arrangement of pores in the scaffolds, exposed surface area and porosity also play a crucial role in the

relationship between cells and matrix. These mechanical properties of Matrigel samples exhibit notable heterogeneity [113]. Additionally, animal-derived cellular matrices raise ethical and clinical concerns in the context of eventual transplantation of human organoids due to their immunogenic potential [114]. The various specific applications of Matrigel-based organoids have been discussed exhaustively [115,116]. In view of these limitations, some of the protocols have dispensed with Matrigel altogether. Alternative methods for culturing Matrigel-independent organoids have also emerged. These include the use of ECMs derived from decellularized tissues and collagen, the application of synthetic polymer-based hydrogels and the incorporation of engineered ECM proteins [117–121].

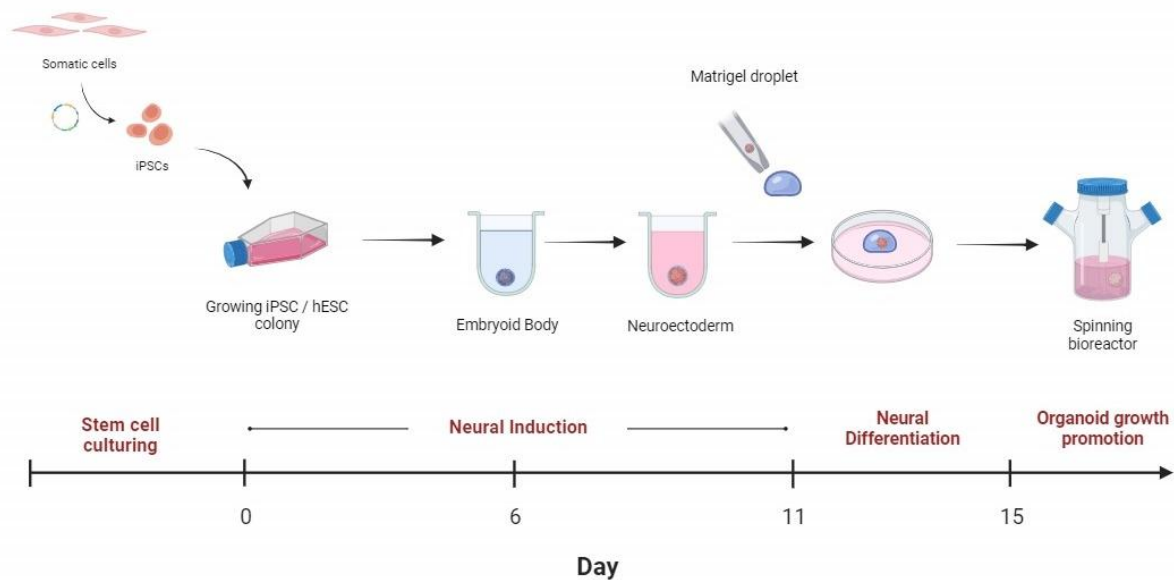


Figure 3. Schematic illustration of the standard organoid formation process. Overview protocol to generate brain organoids with defined differentiated compartments based on Lancaster et al. 2013 [79]. The process begins by seeding 4,500 iPSCs into round-bottom ultra-low-adhesion 96-well plates, creating spherical structures called embryoid bodies (EBs) in a culture medium appropriate for growth. After 6 days, EBs are transferred to low-adherence 24-well plates in neural induction medium for the next 5 days giving rise to neuroectodermal tissue. The individual tissues are then encapsulated in separate Matrigel droplets. Once the Matrigel is solidified, tissue in droplets is supplemented with differentiation medium and maintained in stationary culture conditions for 4 days. Subsequently, Matrigel droplets containing the tissues are placed in a rotating bioreactor along with a differentiation medium designed to promote long-term viability of the organoids. This configuration facilitates the diffusion of oxygen, nutrients, and waste, thus contributing to the continued health and development of the organoids. The tissues are then transferred individually to separate Matrigel droplets. Created with BioRender.com

4.1 Pluripotent Stem Cells used to model AD

Human AD brain organoids have been obtained from iPSCs that harbor genetic mutations found in FAD or the risk factor for the onset of SAD, the APOE4 allele. In the former, iPSCs derived from patients with genomic mutations in the PSEN1, PSEN2 or APP genes. In particular, 3D neural cultures derived from iPSCs with the PSEN1 E9 mutation or the APP K670N/M671L variant

more faithfully recapitulate AD pathology including early amyloid plaque formation and synaptic dysfunction [122]. In turn, SAD patients carrying the APOE4 variant have alterations in lipid metabolism, impaired A β clearance and tau protein dynamics that exacerbates the formation of A β plaques and P-tau, reflecting the complex interplay between genetic risk factors and AD pathology [123–125].

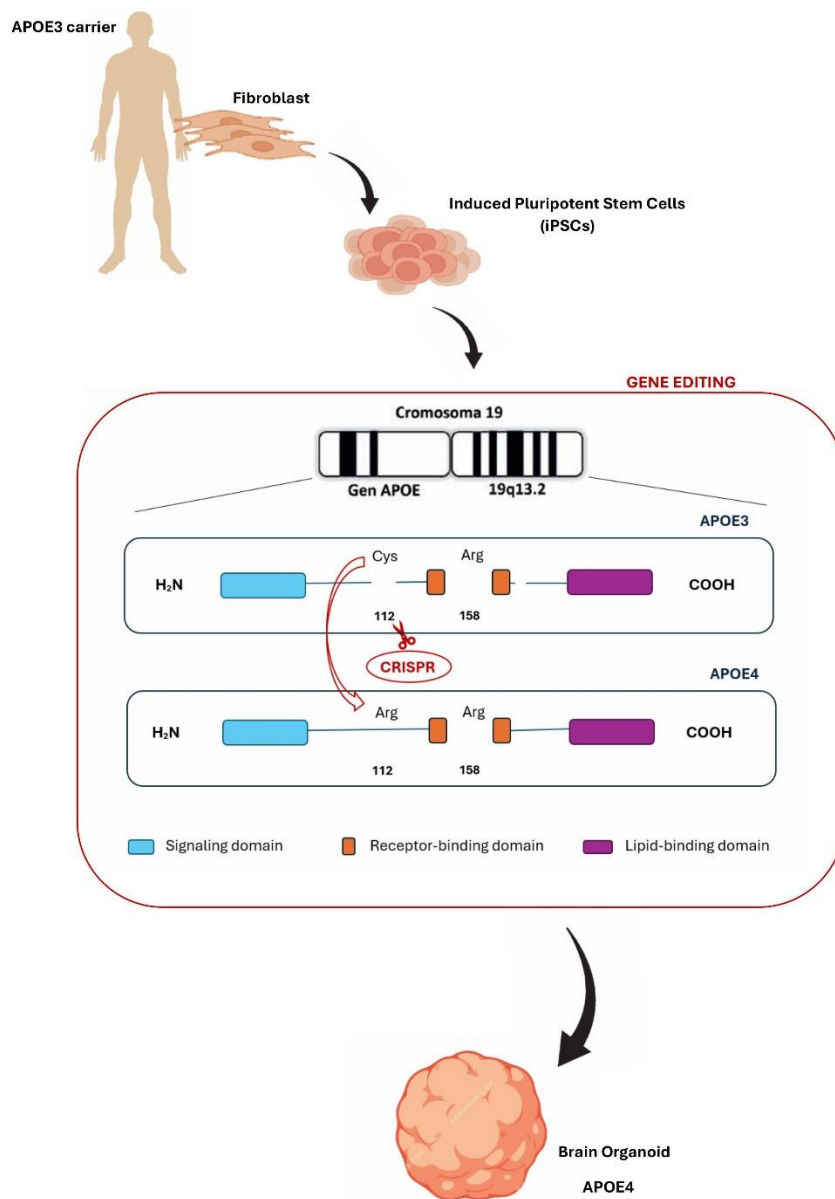


Figure 4. Generation of isogenic APOE4 cerebral organoids for Alzheimer's Disease modeling and drug screening. Fibroblasts from a patient with the APOE3 allele are genetically engineered using CRISPR/Cas9 technology to convert the sequence into APOE4, a variant associated with an increased risk of AD. A plasmid vector containing a specific guide RNA and a repair template encoding the Arg112 mutation is used to induce homology-directed repair after Cas9-mediated cleavage. The edited cells are reprogrammed into iPSCs and differentiated into cerebral organoids for comparative analysis of the phenotypic effects between APOE3 and APOE4 variants. These isogenic cell lines can be employed to investigate the differential accumulation of amyloid-beta and tau proteins, assess neuroinflammatory responses, and study synaptic dysfunction associated with AD. Organoids from these cell lines provide a platform for high-throughput drug screening to identify compounds that may counteract the detrimental effects of the APOE4 allele, and for exploring gene therapy strategies aimed at correcting or mitigating the impact of APOE4 in a patient-specific context. Moreover, these models can contribute to understanding how APOE4 influences the blood-brain barrier integrity and lipid metabolism, providing insights into the systemic aspects of AD pathology. Created with BioRender.com

Moreover, the production and distribution of APOE in the CNS are not uniform across different cell types, which can have important implications for organoid modeling. Astrocytic APOE constitutes the major fraction of brain APOE, accounting for approximately 75–80% of total levels and is predominantly secreted into the extracellular space. In contrast, neuronal APOE, which accounts for about 15–20% of brain APOE, remains largely intracellular [126–128]. The differential contribution of these cell types to overall APOE levels and their secretion patterns is crucial for accurately modeling APOE-related pathology in organoids. To ensure the presence of astrocytes in organoids, it is important to note

that mature astrocytes typically emerge after approximately 2.5 to 3 months of development, as indicated by the expression of markers such as GFAP (glial fibrillary acidic protein) and S100 β (S100 calcium-binding protein- β) [129]. As previously mentioned, these astrocytes play a critical role in the overall APOE production, significantly contributing to the extracellular levels of APOE in the CNS. Understanding these dynamics is essential for guiding the design of organoids that more accurately represent the physiological and pathological roles of APOE, including how its dysregulation might contribute to AD. This consideration is particularly relevant when employing gene-editing

techniques, such as CRISPR/Cas9, to introduce specific mutations or manipulate APOE expression in organoid models (Fig. 4).

In human AD, tau hyperphosphorylation is a multifaceted process influenced by a range of factors, including genetic predisposition and complex interactions between tau and A β [130]. Under physiological conditions, tau undergoes cycles of phosphorylation and dephosphorylation, regulated by kinases and phosphatases that maintain its function. In contrast, in AD, an imbalance occurs, leading to excessive proline-directed phosphorylation driven by kinases such as cdk5, GSK3, and calcium calmodulin kinase II, which disrupts tau function and promotes aggregation. Furthermore, proteins responsible for dephosphorylation, such as phosphatase 2, fail to maintain the balance, resulting in tau hyperphosphorylation [131,132]. Tau can be

phosphorylated at various sites, with each site potentially affecting its aggregation properties differently, depending on the specific amino acid modified [133]. It should be noted that, while phosphorylation promotes tau aggregation, it is not strictly required for it. Modeling tau pathology in iPSC-derived brain organoids is challenging due to this complexity. Although these organoids offer a promising platform for AD studies, they often fall short in accurately replicating the diverse tau pathology observed in human brains. Specifically, these organoids frequently lack the full spectrum of tau isoforms present in human neurons, which affects tau oligomerization and fibrillization properties. The absence of these isoforms can lead to discrepancies in the formation and characteristics of tau aggregates compared to those seen *in vivo* [134].

Table 1. Summary of studies that have developed Alzheimer's disease (AD) models using cerebral organoids derived from patient induced pluripotent stem cells (iPSCs).

FAMILIAL ALZHEIMER'S DISEASE (FAD) MUTATION		
PUBLICATION	IPSC CULTURING	PHENOTYPE
Raja et al. 2016 [143]	iPSCs from FAD patients (APP duplication; PSEN1 mutation); iPSCs of patients with duplication in the APP gene and mutation in the PSEN1 gene.	Robust and relevant phenotypes similar to AD. Large numbers of intra- and extracellular A β aggregates and significant P-tau immunoreactivity.
González et al. 2018 [144]	IPSCs of patients with duplication in the APP gene and mutation in PSEN1.	A β and P-tau aggregates, with a degree of cellular apoptosis proportional to the accumulation of protein aggregates. Over time, structures resembling extracellular senile amyloid plaques and intracellular NFTs associated with late-onset AD.
Ghatak et al. 2019 [145]	iPSCs from AD patients with APP Swedish mutation. iPSCs from AD patients with PSEN1 mutation.	Enhanced neuronal hyperexcitability; increased spontaneous firing rates; decreased neurite length; altered synaptic density, and aberrant calcium dynamics.
Kuehner et al. 2021 [146]	FAD patient-derived iPSC lines: PSEN1 Y155H; PSEN1 M139V; PSEN1 intron4 deletion and APP V717I	Aberrant 5-hydroxymethylcytosine (5hmC) regulation and altered DNA methylation patterns.
Yin et al. 2021 [147]	IPSCs from AD patients with PSEN2 ^{N141I} mutation.	Higher A β 42/A β 40 ratio, asynchronous calcium transient and enhanced neuronal hyperactivity.
Vanova et al. 2023 [148]	iPSC lines from AD patients with PSEN1 (A246E) and PSEN2 (N141I) mutations.	Defective tissue patterning and altered development; irregular layering and disrupted cytoarchitecture; delayed neuronal, and glial cell maturation.
Holubiec et al. 2023 [149]	iPSCs from patient-derived fibroblasts carrying the Swedish mutation in APP (APP ^{sw}).	Increased oxidative stress; elevated ROS production; mitochondrial membrane damage; dysfunction in mitochondrial respiratory chain, and ATP production.
SPORADIC ALZHEIMER'S DISEASE (SAD) MUTATION		
PUBLICATION	IPSC CULTURING	PHENOTYPE
Lin et al. 2018 [150]	Isogenic iPSC lines harboring homozygous APOE4 alleles.	A β aggregates and tau hyperphosphorylation; synaptic deficits; lipid accumulation, and disruptions in cellular signaling pathways associated with AD phenotypes.
Zhao et al. 2020 [151]	IPSCs derived from AD patients carrying APOE ϵ 4/ ϵ 4.	A β aggregates; phosphorylated tau; synapse loss, and apoptosis.

Zhao et al. 2020 [152]	iPSCs derived from patients with APOE4 genotype.	Disrupted lipid homeostasis and greater accumulation of α -synuclein aggregates.
Park et al. 2021 [153]	iPSCs from patients with APOE4 mutation.	A β 40/42ratio ; tau accumulation ; P-tau, and calcium transients.
Hernández et al. 2021 [154]	iPSCs from individuals with an APOE ϵ 4/ ϵ 4 genotype.	APOE levels; A β 40/42 ratio, and P-tau.
ALTERNATIVE PROTOCOLS		
PUBLICATION	IPSC CULTURING	PHENOTYPE
Pavoni et al. 2018 [137]	Primary fibroblast obtained from ATCC (CRL-2522) were reprogrammed into iPSC. No specific mutations. Small-molecule induction of AB-42 production.	Accumulation of A β -42 peptide; plaque-like structures, and neuronal dysfunction.
Alic et al. 2020 [141]	iPSCs derived from patients with Trisomy 21 (DS).	Increased A β -42; P-tau; amyloid plaques; BACE2 as a gene dose-sensitive suppressor; neurogenesis defects and altered brain development.
Chen et al. 2021 [142]	iPSCs derived from individuals without known familial genetic mutations. Cerebral organoids were exposed to human serum to induce SAD features.	Increased production of A β -42; accumulation of hyperphosphorylated tau; synaptic loss, and impaired neural network.

The studies included cover familial AD mutations, mutations related to SAD, as well as other alternative methods used to induce neurodegeneration in these models. The table details the phenotypic characteristics observed in the organoids, such as A β accumulation, P-tau presence, and other pathological markers associated with AD. Although recent studies have emphasized the critical role of astrocytes in AD pathogenesis, it is notable that many organoid models fail to address or study astrocyte-related phenotypes. This oversight could limit the understanding of the full spectrum of neurodegenerative processes in AD, as the involvement of astrocytes is increasingly recognized as a significant factor in disease progression.

Furthermore, gene-editing techniques such as CRISPR/Cas9 and viral vectors like AAV-mediated gene transfer, have been employed to introduce tau mutations like tau-P301L to promote tau aggregation in organoids [135]. However, these mutations are commonly associated with other tauopathies rather than AD and may therefore provide insights into general tau aggregation mechanisms instead of AD-specific tau pathology.

Recent advancements using astrocyte-neuron spheroids have shown potential in modeling tau propagation accurately. In these models, P-tau can spread from pre-treated to untreated neurons, providing a closer approximation of tau's self-propagation in a more complex cellular environment [136].

Additional research addresses AD pathology by exploring alternative signaling pathways (Table 1). Using the compound Aftin-5, Pavoni et al. managed to chemically induce an AD model, triggering a notable increase in A β production in organoids derived from healthy patients [137]. In another study, Cairns and colleagues investigated the potential role of herpes simplex virus type 1 (HSV-1) in promoting AD-related pathologies in neural spheroids cultured on porous silk scaffolds [138].

This line of inquiry stems from pioneering work by the Itzhaki laboratory, which revealed significantly elevated levels of Herpesviridae DNA in amyloid plaques extracted from postmortem brain tissue of patients with sporadic AD [139]. A recent investigation demonstrated that brain organoids infected with HSV-1 faithfully reproduced the neuropathology associated with the accumulation of A β plaques [140]. This included the

formation of multicellular A β deposits, alteration in the regulation of endogenous mediators related to AD, reactive gliosis, the presence of neuroinflammation and neuronal loss [140]. Furthermore, the inhibition of the mitochondrial enzyme called pitrilysin metalloproteinase 1 (PITRM1) resulted in a significant increase in the aggregation of A β , P-tau and the appearance of neuronal apoptosis in cortical organoids derived from iPSCs [99]. Overall, these alternative models provide unique advantages and mechanisms to characterize AD without the genetic modifications associated with FAD or SAD [137,141,142].

4.2 Limitations related to epigenome preservation

Recent advancements in AD modeling have brought to light the importance of preserving epigenetic integrity, revealing key limitations of current methodologies. While iPSCs have revolutionized disease modeling by enabling the generation of various human cell types, they present challenges, particularly the loss of age-related epigenetic signatures upon reprogramming somatic cells. This cellular rejuvenation can obscure the age-dependent aspects of neurodegenerative diseases like AD [155–159], limiting the ability of iPSC-derived models to fully capture relevant aging processes and disease phenotypes [160–162].

In light of these limitations, recent research has explored alternative methodologies that preserve epigenetic fidelity. Mertens et al. (2022) elucidated that neurons directly induced from fibroblasts of AD patients retain critical disease-related epigenetic modifications,

which are lost when fibroblasts are reprogrammed into iPSCs [163]. Their findings emphasize that these epigenetic features are crucial for understanding AD pathology and are preserved in models bypassing iPSC reprogramming. Following this approach, Sun et al. (2024) introduced direct neuronal reprogramming techniques combined with 3D spheroid cultures, offering a more accurate model of AD neuropathology while conserving disease-relevant epigenetic attributes [164]. The retention of these modifications in 3D models provides a more nuanced understanding of disease progression. Together, these studies emphasize the necessity of preserving epigenetic integrity to accurately model AD mechanisms.

4.3 Vasculature deficiency in the context of AD

Despite the impressive tissue architecture provided by the three-dimensional structure of brain organoids, a significant obstacle arises in the form of nutrient and oxygen deficiency in the organoid's core due to the lack of microvasculature [165]. Certain cells, such as radial glia, can maintain their viability even in the innermost layers of the organoid. This may be due to their unique metabolic requirements and their elongated morphology, which allow them to interact with the external environment. However, as the neuronal population differentiates and increases, these cells are gradually displaced inward and experience necrosis due to the lack of nutrients. Significant advances have been made in improving the survival of brain organoids, including the administration of growth factors such as BDNF, as well as the transplantation of organoids into a rodent host [166,167]. Transplantation has not only demonstrated unprecedented cellular survival due to effective blood circulation, but it has also shown the neuronal connectivity of the organoid with the rodent brain [167]. These successful *in vivo* transplants suggest the possibility that brain organoids have an intrinsic potential to establish functional connections. However, this is a labor-intensive strategy requiring specialized skills, making it difficult to conduct comprehensive characterization across multiple samples. To address this challenge in a fully *in vitro* system, a traditional organotypic slice culture technique has recently been employed [168]. This involves air-liquid interface (ALI) cell culture, which leads to substantial improvements in neuronal maturation and survival as it shows a higher number of cortical neuron populations and a decrease in cell death [169]. Additionally, the expression of human ETS variant 2 transcription factor (hETV2) reprograms human dermal fibroblasts into ECs in the absence of growth factors and induces the formation of vascular-like structures in organoids [170]. This model offers a valuable

platform that reduces the apoptotic and hypoxic condition of organoid core. Alternatively, iPSC-derived ECs vascularize developing brain organoids when adhered to their surface [171]. In brief, ECs embedded together along with brain organoids in a Matrigel basement membrane matrix generate tubular structures with CD31+ cells with capillary structures weeks later [171]. Vascularized 3D spheroid models also include a microfluidic chip (Fig. 5). From the microchannels of these devices, angiogenic sprouts or blood vessel-like structures are induced towards the spheroid to form a continuous lumen capable of delivering biological substances to the interior of the organoid [172].

The study by Wang et al. (2021) unveiled significant advancements in the culture of brain organoids using microfluidic chips, enhancing several critical aspects of cellular development. In particular, they observed increased cellular proliferation, homogeneous differentiation of more neural progenitors into neurons and glia, and preservation of cellular heterogeneity resembling *in vivo* conditions. The chips also reduced hypoxia-related stress and apoptosis. Additionally, organoids showed improved synaptic activity and electrophysiological patterns, indicating greater neuronal maturity and functionality [173]. These findings highlight the considerable potential of microfluidic chips to enhance the quality, complexity, and functionality of brain organoids, opening new avenues in biomedical research.

4.4 Lack of blood-brain barrier

Despite the advances in vascularizing brain organoids, replicating the intricate structure and function of the BBB remains challenging. Recent developments made feasible the integration of astrocytes and pericytes, which are essential for BBB integrity, into brain organoids. In these advanced models, BBB spheroids exhibit high expression of tight junction proteins on their surface, along with VEGF-dependent permeability, efflux pump activity, and receptor-mediated transcytosis of angiopep-2 [174–176]. Therefore, this setup successfully replicates key aspects of BBB functionality, enabling accurate assessment of drug permeability and efficacy. Additionally, these models are valuable for studying AD, as BBB leakage is a well-known risk factor for the condition [177–181]. By exposing brain organoids to human serum, researchers can mimic the consequences of BBB breakdown observed in AD patients [142,182]. This approach has been shown to recapitulate AD-like pathologies, including increased A β aggregates, elevated levels of p-tau, synaptic loss, and impaired neural networks. This exposure also induces elevated levels of beta-secretase 1 (BACE) and glycogen synthase kinase-3 α/β (GSK3 α/β), further

elucidating the mechanisms underlying AD [142]. In turn, microfluidic devices offer a more dynamic and realistic representation of the BBB by introducing blood flow and simulating the shear stress that occurs at the barrier. These advancements enhance the physiological relevance of BBB models in culture, providing a closer approximation

to in vivo conditions. However, a significant limitation of these systems is their requirement for specialized equipment and technical expertise, which hampers their broader application within the neuroscience research community.

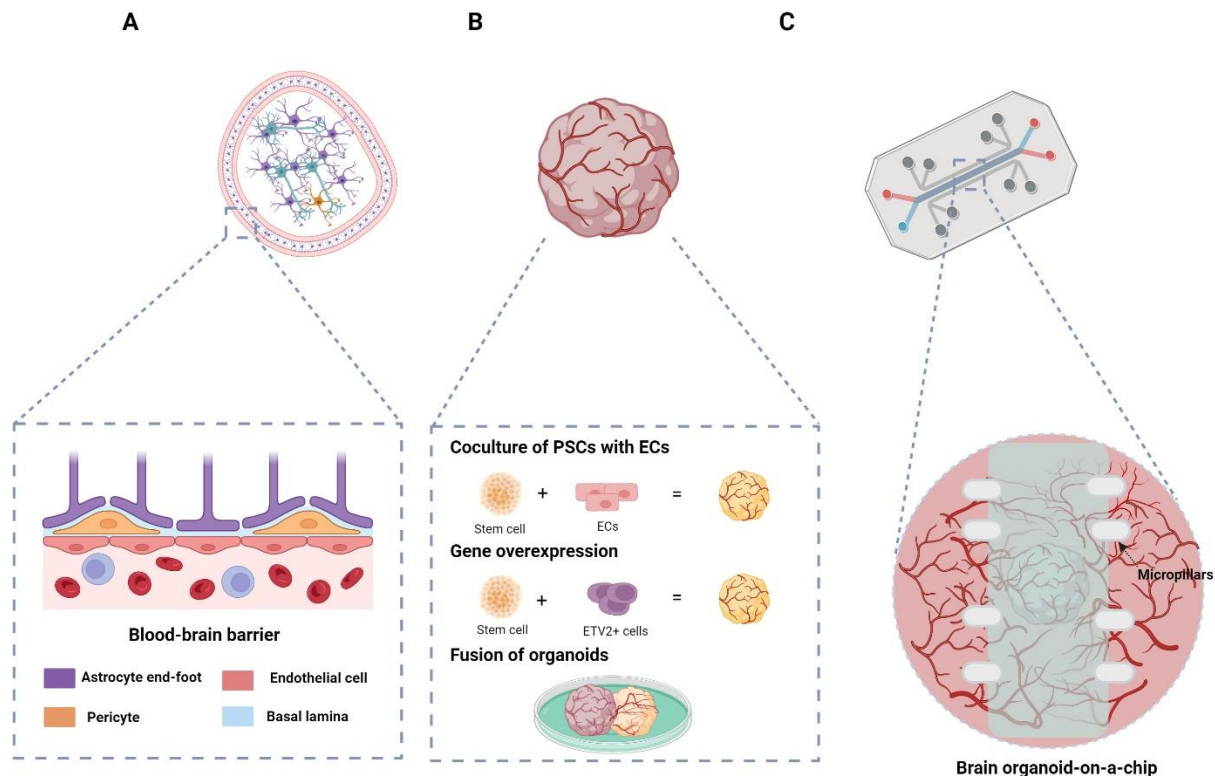


Figure 5. Strategies to alleviate hypoxia and metabolic stress in brain organoids: Blood-Brain Barrier models, vascularization, and microfluidic integration. In brain organoids, as mentioned in the text, the central regions often experience limited glucose and oxygen supply due to the dense cellular architecture, leading to a hypoxic environment [165]. This scarcity triggers the activation of the Warburg effect, where cells shift from oxidative phosphorylation to anaerobic glycolysis to produce energy. Although less efficient, this metabolic adaptation allows cells to survive by rapidly consuming glucose and producing lactate, even in the presence of oxygen. However, the accumulation of lactate and the acidic microenvironment can lead to cellular stress, apoptosis, and eventual necrosis in the organoid's core. To address these challenges, the incorporation of BBB models (A), the introduction of blood vessels formed from endothelial cells (ECs) (B) and microfluidic chips (C), offer promising solutions. These approaches enhance nutrient and oxygen distribution, reduce hypoxia, and limit the reliance on the Warburg effect, thereby preserving cellular viability, supporting complex tissue architecture, and mimicking more accurately the in vivo conditions of the human brain. Created with BioRender.com.

4.5 Lack of immune system

Upon injury, microglia rapidly extend their processes towards the affected area, migrating to the lesion site to recognize pathogens, ramify, and initiate an immune response [183]. In AD brains and mouse models, microglia are frequently observed in close proximity to amyloid plaques, exhibiting an 'activated' proinflammatory phenotype [184,185]. Initially believed to contribute to amyloid deposition, subsequent electron microscopy studies revealed that microglia can engulf A β peptides through endosome-like compartments [186,187].

In vitro experiments utilizing radioisotope or fluorescent-labeled A β and direct injection of fibrillar A β into rodent brains have further demonstrated microglia's capability to internalize the peptide [188–190]. Integrating microglia into brain organoids enhances the capability to model debris clearance and study inflammatory responses. A recent preprint illustrates how interactions between microglia and astrocytes within a 3D neurosphere effectively reduce A β toxicity. Additionally, this study shows that iPSC-derived primitive macrophages, resembling microglia, significantly reduce A β aggregation and promote neuronal survival during A β

treatment in a human 3D neurospheres. These findings suggest that the activation state of microglia-like cells remains consistent across various model systems and underscores the importance of modeling interactions with peripheral immune cells to elicit robust pro-inflammatory responses.

The regional progression of brain atrophy in AD is strongly correlated with tau accumulation [191-193]. In this context, Chen et al. (2023) investigated the role of T cells in neurodegeneration observed in tauopathies. Their study reveals that microglia are essential in recruiting T cells to the brain in response to tau aggregation [194]. Infiltration of T cells exacerbates neurodegeneration and promotes a pro-inflammatory environment. By blocking T cell entry or activation, the researchers observed a significant reduction in neurodegeneration in mouse models of tauopathy. These findings suggest that targeting T cells could offer a novel therapeutic approach for mitigating neurodegenerative diseases like AD, presenting an alternative to traditional treatments focused on tau or microglial dysfunction.

Building on these findings, another study developed a sophisticated 3D human tri-culture system comprising neurons, astrocytes, and microglia derived from iPSCs [195]. This model successfully recapitulates key aspects of AD pathology, including A β aggregation, tau hyperphosphorylation, and neuronal death. Within this system, microglia exhibit an activated phenotype, characterized by increased expression of pro-inflammatory cytokines and enhanced phagocytic activity. Interestingly, while iPSCs-derived microglia play a beneficial role by clearing amyloid plaques and other debris, they also contribute to neuroinflammation and neuronal damage through the release of pro-inflammatory cytokines [195]. This dual role highlights the complexity of microglial function in AD and the importance of targeting their activation states to mitigate harmful effects while enhancing protective functions.

Recent studies have also explored the role of TREM2, a key receptor involved in microglial function, within organoids. For example, it has been demonstrated that brain organoids with TREM2-deficient microglia exhibit impaired phagocytic activity and altered immune responses, exacerbating AD pathology [196]. This highlights TREM2's critical role in regulating microglial function and emphasizes the utility of organoid models in elucidating the molecular mechanisms underlying microglia-mediated contribution to neurodegenerative diseases like AD

4.6 Lack of myelination

A decline in myelination and the subsequent impairment of oligodendrocyte-axon signaling are increasingly

associated with neurodegenerative disorders, such as amyotrophic lateral sclerosis (ALS), PD and AD [197–199]. Hypomyelination also manifests in various neurodevelopmental conditions like DS and is characteristic of leukodystrophies [200,201]. Current efforts focus on enhancing OL function and survival, including the delivery of exogenous oligodendrocyte progenitor cells (OPCs). These approaches aim to restore axonal integrity and neurological function, with potential applications in AD research. However, significant challenges remain in achieving effective differentiation and maturation of OLs in brain organoids, where conventional methods often fail to support their development [202].

A recent study achieved relative robust induction of oligodendrogenesis and myelination, albeit after a prolonged glial differentiation protocol for 210 days [203]. Such extended differentiation timelines make experimentation difficult and impractical. To address this, alternative methods have been developed to accelerate OL specification in organoids by shortening the protocol to 12 weeks of culture [204]. The addition of PDGF-AA and IGF-1 similarly achieved mature OLs by day 100 [205]. Additionally, innovative and simplified one-step protocols for generating human cortical brain organoids further streamlined myelinating oligodendrocyte generation to within just 42 days. However, the organoids exhibited only a few axons with compacted myelin sheaths [206]. This limited myelination may improve with more prolonged culture times or the addition of myelination-enhancing molecules.

Considering that the APOE4 gene is associated with increased susceptibility to demyelination, the study of myelinated organoids could be particularly promising for investigating these complex dynamics in AD. Recent studies have shown that the presence of APOE4 in astrocytes induces significant disruption in lipid balance, affecting essential cellular functions and increasing the risk of demyelination [207–209]. On the other hand, treatment with docosahexaenoic acid has shown potential in promoting remyelination. DHA can protect and restore damaged myelin in experimental models, suggesting it may be useful in counteracting the demyelinating effects of APOE4 [210]. Thus, the innovation in accelerating OL specification and myelination in organoid models is not only a technical advancement but also a pivotal step towards unraveling the complexities of myelination and developing targeted therapies for demyelinating diseases.

Future prospects

While brain organoids derived from human iPSCs have markedly enhanced our capacity to model neurodevelopmental processes and investigate

neurodegenerative diseases such as AD, it is essential to recognize the inherent limitations of these culture systems. These constraints bear significant implications for the accuracy, reproducibility, and translational relevance of the insights gleaned from such models.

One of the most notable limitations is the insufficient functional maturity exhibited by brain organoids. These organoids can faithfully recapitulate critical aspects of early human neurodevelopment, encompassing the genesis, proliferation, and differentiation of neural progenitors into neurons and glial cells. However, they frequently fall short of replicating the complex, multilayered architecture and the full spectrum of functional characteristics inherent to adult human brain tissue [105, 108, 211–213]. This developmental immaturity poses significant challenges for modeling late-onset neurodegenerative diseases, where age-related processes are central to disease pathogenesis. Considering that aging is the primary risk factor for AD, the extent to which brain organoids can be utilized to model age-related neurodegeneration remains uncertain. This underscores the need for rigorous validation of disease-relevant phenotypes. To address these limitations and enhance their relevance for AD research, strategies such as integrating brain organoids with models that simulate neuroinflammatory conditions or BBB dysfunction, as mentioned earlier, may be useful. Additionally, the use of chronic exposure to amyloidogenic peptides, neurotoxins, or stressors that mimic aging processes within organoids may help to induce features more representative of the disease's late-onset nature. These strategies could provide a more accurate platform for investigating AD pathology and testing potential therapeutic interventions. However, as indicated, thorough validation will be necessary to confirm that the disease phenotypes are faithfully reproduced.

In addition to the challenges associated with functional maturity, another critical aspect to consider with brain organoids is their intrinsic heterogeneity. The generation of organoids often results in variability in size, cellular composition, and structural organization [102]. This heterogeneity can lead to inconsistencies in experimental outcomes, thereby complicating the reproducibility and interpretation of results, particularly in studies aimed at modeling complex diseases like AD. It is therefore essential to develop refined protocols promoting uniform organoid formation. Reducing variability through the selection of guided differentiation methods could further enhance the consistency and reliability of organoid-based models. Moreover, as previously mentioned, the use of complex ECMs substitutes, like Matrigel, significantly contributes to this variability. Although Matrigel provides a versatile and affordable scaffold for organoid culture, its inherent

complexity with over 1800 unique proteins, introduces a level of unpredictability [214]. The undefined nature of Matrigel complicates the identification of specific signals necessary for consistent organoid structure and function, and this challenge is further exacerbated by lot-to-lot variations. Such inconsistencies underscore the importance of exploring more defined or synthetic alternatives that could offer a more controlled microenvironment. In addition, organoids remain intrinsically reductionist models as they partially replicate key aspects of brain physiology, such as myelination, microglial activity, and vascularization. The absence of these crucial elements constrains the organoids' capacity to emulate fully the complex in vivo environment of the human brain. As discussed in this review, current research is addressing these gaps by integrating microglial cells and vascular networks through advanced bioengineering techniques, as well as protocols that promote OL maturation. The incorporation of microglia, in particular, is anticipated to facilitate the regeneration of myelin since recent findings demonstrate that its absence can lead to CNS demyelination with increasing age [215]. This highlights the potential for this cell type integration to support myelin repair and improve the accuracy of organoid models in studying AD. Similarly, the incorporation of a functional vascular network into brain organoids, though presenting considerable challenges, is essential for enhancing their physiological fidelity. The absence of a functional vasculature not only restricts nutrient and oxygen diffusion but also omits critical neurovascular interactions that influence neuronal function, glial support, and BBB integrity. Thus, methods such as spinning bioreactors, the induction of ECs and perivascular cells, and the integration of models that replicate the BBB, including microfluidic chips, are being explored.

The fusion of organoids to create "assembloids" offers a promising approach to overcome the limitations of traditional, reductionist brain models. In particular, a cortical-blood vessel assembloids that successfully replicated AD phenotypes was developed [216]. By integrating cortical organoids with vascular elements, the model allowed for the study of critical neuron-glia-vascular interactions. Following SARS-CoV-2 infection, these assembloids exhibited glial activation and other AD-like features, demonstrating their utility in exploring complex disease mechanisms and potential therapeutic targets. Moreover, assembloids can also be engineered to replicate the intricate neuronal connections implicated in AD that alter large-scale brain networks, particularly those involved in memory formation [217,218]. These changes encompass a deceleration of the alpha rhythm toward theta frequencies, aberrant gamma activity, and a reduction in slow-wave oscillations during sleep [219–

221]. It would be particularly compelling to leverage advanced tools such as multi-electrode arrays within assembloid models to investigate these specific electrophysiological disruptions. This approach could offer significant insights into how hyperactivity in local networks, along with impaired connectivity between key brain regions such as the hippocampus and the cortex, contributes to the cognitive dysfunction observed in AD pathology [136]. Furthermore, assembloid models provide a promising platform for studying the propagation of tau protein across neuronal circuits, particularly its spread from the entorhinal cortex to the hippocampus and other neocortical areas [222–224]. Understanding these mechanisms is crucial for unraveling the progression of tau pathology and its role in driving the disease. Following this line of thought and considering the emerging hypothesis that gut-derived infective agents may influence AD progression, incorporating gut-brain assembloids could be highly informative. These gut-brain assembloids could facilitate the investigation of how pathogenic microbes or inflammatory signals originating in the gut might contribute to AD pathology. Such models could provide new insights into the gut-brain axis and its role in neurodegeneration, particularly in understanding how gut-derived infections or dysbiosis could trigger or exacerbate amyloid plaque formation and tau pathology in the brain.

From a practical perspective, brain organoids require significant time to develop and mature, which can increase costs and reduce experimental efficiency. Therefore, exploring methods to accelerate this maturation process is crucial. For example, NOTCH inhibitors effectively speed up neuronal differentiation in vitro and conceivably in brain organoids [225]. Additionally, ensuring that all cells within the organoid reach a similar level of maturity is vital for accurate disease modeling. Techniques such as single-cell RNA sequencing may help verify this uniformity. Specifically, it is important to monitor gene expression in astrocytes in AD organoids as these cells are the primary cells expressing APOE in the adult brain. Proper expression of these genes is crucial for studying AD and ensuring that organoids accurately model late-onset pathology.

In conclusion, while brain organoids have advanced our understanding of AD, significant challenges remain. Nevertheless, with ongoing improvements in functional maturity, uniformity, and the incorporation of key physiological elements, we anticipate that these models will become increasingly robust. Overcoming these challenges will enable the development of more accurate platforms for investigating AD and testing therapeutic interventions, ultimately bringing us closer to effective treatments.

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